(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 451 867 A1**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **91105881.6**

(22) Date of filing: **12.04.91**

(51) Int. Cl.5: **C07K 7/10**, C07K 1/04, A61K 37/02, A61K 37/43

The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(30) Priority: **12.04.90 JP 96952/90**

(43) Date of publication of application: **16.10.91 Bulletin 91/42**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IT LI LU NL SE**

(71) Applicant: **MITSUBISHI KASEI CORPORATION**
5-2, Marunouchi 2-chome Chiyoda-ku
Tokyo 100(JP)

(72) Inventor: **Kanmera, Tatsuhiko**
1159-2, Ichigaocho, Midori-ku
Yokohama-shi, Kanagawa-ken(JP)
Inventor: **Mori, Akihisa**
2-11-1, Naruse
Machida-shi, Tokyo(JP)
Inventor: **Nakao, Yoshihide**
2-10-2, Ogawa
Machida-shi, Tokyo(JP)
Inventor: **Minegishi, Toshihiko**
28-6, Umegaoka, Midori-ku
Yokohama-shi, Kanagawa-ken(JP)

(74) Representative: **Hansen, Bernd, Dr. Dipl.-Chem. et al**
Hoffmann, Eitle & Partner Patent- und Rechtsanwälte Arabellastrasse 4 Postfach 81 04 20
W-8000 München 81(DE)

(54) **Parathyroid hormone antagonists.**

(57)

Peptide derivatives of the following general formula (I):

```
AAA BBB CCC DDD EEE FFF His GGG HHH Gly Lys Ser Ile Gln Asp Leu
 1               5                  10                  15

Arg Arg Arg Phe Phe Leu III JJJ Leu Ile Ala Glu Ile His Thr Xaa
            20                  25                  30
```

... (I)

wherein AAA is deletion or Ser, BBB is deletion or Glu, CCC is deletion, Ile, Phe, Leu, cyclohexylalanine, D-alle or Lys substituted at the ϵ-position by C_6-C_{18} alkylcarbonyl group, DDD is Gly or Gln, EEE is Leu, Nle or Phe, FFF is Met, Leu or Nle, GGG is Ala, Ser, Leu, Asn, Asp or Gln, HHH is Leu, Glu or Lys, III is His, Lys or Arg, JJJ is His, Lys or Arg, Xaa is Ala modified at the carboxy terminal with an amino group, provided that CCC and DDD may independently be modified at the amino terminal by C_2-C_{18} alkylcarbonyl group, and that EEE is not Leu when FFF is Met, GGG is Asn, HHH is Leu, III and JJJ are His, and the salts thereof, which exhibit a potent inhibitory activity against hPTH and are useful as a therapeutic agent for treating dysbolism associated with calcium or phosphoric acid, such as hypercalcemia, osteoporosis, hyperparathyroidism, renal osteodystrophy, and the like, and other related diseases involving PTH or PTHrP.

EP 0 451 867 A1

The present invention relates to novel parathyroid hormone antagonists.

Parathyroid hormone (PTH) is an important hormone to take charge of the calcium metabolism in living bodies. Recently, analogous peptides having an activity similar to PTH, referred to as "parathyroid hormone related peptides" (PTHrP), have been discovered. Human PTHrP is a polypeptide consisting of 141 amino acids, and its PTH-like biological activities, namely elevation of blood level of calcium, acceleration of bone resorption, lowering of blood level of phosphorus, lowering of urine level of calcium, increase of urine level of cAMP, and renal activation of hydroxylase at the 1-position of vitamin D, have recently been reported [Horiuchi, et al., Science, Vol. 238, 1988; Kemp, et al., Science, Vol. 238, 1988].

It is known that PTH fragments which lack several amino acids at the amino terminal and carboxy terminal of PTH, such as PTH (3-34), PTH (7-34) or their derivatives inhibit the PTH action, and they are useful as PTH antagonists. Similar inhibitory action has also been reported on PTHrP fragments [Suva et al., Science, Vol. 237, 893 (1987); Rabbani et al., Report at the Meeting of American Bony Metabology 1988].

European Patent Publication No. 341,962 discloses, as a human humoral hypercalcemic factor (hHGF), PTH derivatives such as hPTHrP(8-34)NH_2, hPTHrP(14-34)NH_2, and the like. However, they show insufficient activity for clinical use.

As the result of extensive study for the purpose of obtaining PTHrP derivatives showing more potent PTH antagonistic activity than known PTH or PTHrP derivatives, such as [Tyr^{34}]-hPTH (3-34)-NH2, hPTHrP (3-34)-NH_2 and [Leu^{11},D-Trp^{12}]-hPTHrP(7-34)-NH_2, the present inventors have found that a certain class of PTHrP derivatives possess excellent antagonistic activity against PTH.

Thus, the present invention provides the peptide derivatives of the following general formula (I):

```
AAA BBB CCC DDD EEE FFF His GGG HHH Gly Lys Ser Ile Gln Asp Leu
 1                   5                  10                  15

Arg Arg Arg Phe Phe Leu III JJJ Leu Ile Ala Glu Ile His Thr Xaa
            20                  25                  30
```

... (I)

wherein AAA is deletion or Ser, BBB is deletion or Glu, CCC is deletion, Ile, Phe, Leu, cyclohexylalanine, D-alle or Lys substituted at the ϵ-position by C_6-C_{18} alkylcarbonyl group, DDD is Gly or Gln, EEE is Leu, Nle or Phe, FFF is Met, Leu or Nle, GGG is Ala, Ser, Leu, Asn, Asp or Gln, HHH is Leu, Glu or Lys, III is His, Lys or Arg, JJJ is His, Lys or Arg, Xaa is Ala modified at the carboxy terminal with an amino group, provided that CCC and DDD may independently be modified at the amino terminal by C_2-C_{18} alkylcarbonyl group, and that EEE is not Leu when FFF is Met, GGG is Asn, HHH is Leu, III and JJJ are His, and the salts thereof.

The present invention will be explained in detail below.

The peptide derivatives of the invention are represented by the above-mentioned formula (I), i.e., SEQ ID No. 1. In the definition of the formula (I), specific examples of the C_2-C_{18} alkylcarbonyl group are acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, 3-methylvaleryl (3-methylpentanoyl), heptanoyl, octanoyl, nonanoyl, decanoyl, lauroyl, myristoyl, palmitoyl and stearoyl, and the C_5-C_{18} alkylcarbonyl group includes the above-noted groups which contains from 5 to 18 carbon atoms.

As previously stated, PTH or PTHrP fragments which lack 2-13 amino acid residues at the amino terminal show antagonistic property against PTH or PTHrP. However, such antagonistic activities are generally weak for practical use. Accordingly, development of peptide compounds having more potent antagonistic activity has long been desired. For this purpose, it is a general procedure to substitute one or more amino acids of PTH or PTHrP and then examine the activity of the resultant products. However, it is very difficult to previously estimate the result of particular substitutions. In addition, substitution of plural amino acids does not always bring about additive or synergistic effect expected from the known results in each substitution. Thus, it is far more difficult to previously estimate the results of a combination of two or more substitutions.

Under such circumstances as mentioned above, the inventors of the pesent invention synthesized and investigated a vast amount of PTHrP derivatives and found that the above-mentioned PTHrP derivatives of the formula (I) exhibited very potent antagonistic activity.

One of the key elements of the substitution of the amino acid or acids of PTHrP was introduction of a hydrophobic substituent at the 5- and 8- positions of naturally-occurring PTHrP and also substitution of other amino acid or acids so that the increased antagonistic activity due to the just-mentioned introduction of a hydrophobic substitution may be retained or enhanced. Another key element was introduction of a strong hydrophobic substituent into the N-terminal, which was accomplished by introduction of an amino acid bearing a hydrophobic acyl group into the N-terminal. Such substitution as mentioned above appears effective for both increase of activity and stabilization of the derivatives in a living body.

Illustrative examples of the PTHrP derivatives of the present invention are those as shown hereinafter in Table 2. These derivatives can also be converted to pharmacologically acceptable salts such as hydrochloride, acetate or the like, without loss of the activity.

The abbreviations used in the present specification have the following significances. All the amino acids take L-form, unless otherwise stated.

Asp	: Aspartic acid
Thr	: Threonine
Ser	: Serine
Glu	: Glutamic acid
Asn	: Asparagine
Gln	: Glutamine
Gly	: Glycine
Ala	: Alanine
Met	: Methionine
Met(O)	: Methionine sulfoxide
Ile	: Isoleucine
Leu	: Leucine
Phe	: Phenylalanine
Tyr	: Tyrosine
Lys	: Lysine
His	: Histidine
Arg	: Arginine
Cha	: β-Cyclohexylalanine
Nle	: Norleucine
alle	: Alloisoleucine
Lys(Pal)	: ϵ-palmitoyllysine
Lys(Myr)	: ϵ-Myristoyllysine
Lys(Cap)	: ϵ-Decanoyllysine
Lys(Hex)	: ϵ-Hexanoyllysine
Ac	: Acetyl
Mpa	: 3-Methylpentanoyl
Hex	: n-Hexanoyl
Cap	: n-Decanoyl
Myr	: Myristoyl
Pal	: Palmitoyl
Boc	: t-Butyloxycarbonyl
Z	: Benzyloxycarbonyl
OcHx	: Cyclohexyl ester
OBzl	: Benzyl ester
Bzl	: Benzyl
Bom	: Benzyloxymethyl
Tos	: p-Toluenesulfonyl
Cl-Z	: 2-Chlorobenzyloxycarbonyl
Bop Reagen	t : Benzotriazo-1-yl-tris(dimethylamino)-phosphonium hexafluorophosphate

The PTHrP derivatives (I) of the present invention can be prepared by repeating the condensation reaction of each protected amino acids in the order of amino acid sequence represented by the formula (I), starting from the C-terminal, in accordance with the solid phase peptide synthesis generally adopted, and then subjecting the resultant protected peptides to acid decomposition, aminolysis or known other procedures for removing the protecting groups and solid carrier used. The derivatives can also be prepared by condensing various peptide fragments according to so-called liquid phase synthetic methods. These general synthetic methods are described in detail in various literatures and text books [Izumiya, et al.,

"Fundamentals and Practice of Peptide Synthesis", Maruzen, 1985: Gross & Meienhofer, "The Peptides", Vol. 2, Academic Press, 1980].

Solid carriers usable for synthesizing the peptide of the present invention are those conventionally used for the peptide synthesis. Specific examples of the carriers include substituted benzyl type polystyrene resins, hydroxymethylphenylacetamide type polystyrene resins, substituted benzhydryl polystyrene resins and polyacrylamide resins capable of binding to a peptide. The condensation of amino acids can be attained in a conventional manner used for peptide synthesis, for example, dicyclohexylcarbodiimide (DCC) method, acid anhydride method, activated ester method, or a method using Bop reagent. Protecting groups used for protecting amino acids as starting materials are those already known in the peptide synthesis, which are easily removable by known means such as acid decomposition or the like. Examples of protecting groups for an amino group in the side chain are trifluoroacetyl, benzyloxycarbonyl and substituted benzyloxycarbonyl, such as (ortho- or para-)chlorobenzyloxycarbonyl, (ortho- or para-)bromobenzyloxycarbonyl or the like. Protecting groups for an α-amino group are those comparatively instable to an acid and include t-butoxycarbonyl, t-amyloxycarbonyl, p-methoxybenzyloxycarbonyl or the like. A carboxy group is protected by an ester group. Examples thereof are benzyl ester, substituted benzyl ester, alkyl ester such as cyclohexyl ester, cycloheptyl ester or the like. A guanidino group can be used without protection, or in the protected form with nitro or arylsulfonyl, such as tosyl, methoxybenzenesulfonyl, mesitylenesulfonyl or the like. Tosyl, benzyl, dinitrophenyl, benzyloxymethyl or the like may be used for protecting an imidazole. A hydroxy group of serine and threonine can be used without protection or after protection with benzyl, substituted benzyl or the like. An indole group of tryptophane is used without protection or after protection with formyl or the like.

The final deprotection and separation of the resultant peptide from the solid carrier can be conducted by the use of dry hydrogen fluoride in the presence of various scavengers. The scavengers mean those generally used for peptide synthesis and illustratively include anisole, (ortho-, meta- or para-)cresole, dimethyl sulfoxide, thiocresole, ethanedithiol, mercaptopyridine and the like.

Elongation and deprotection of the resultant peptide can be performed in accordance with FMOC method (Fields et al. Int. J. Pept. Protein Res. 35, 16, 1990). Purification of the peptide may be performed by gel filtration, ion exchange chromatography, reversed phase chromatography under high or low pressure.

Purified peptides can be converted into their salts by gel chromatography equilibrated with an aqueous acid solution.

The PTHrP derivatives (I) of the present invention may be useful as a therapeutic agent for treating dysbolism associated with calcium or phosphoric acid, such as hypercalcemia, osteoporosis, hyperparathyroidism, renal osteodystrophy, and the like, and other related diseases involving PTH or PTHrP.

The following detailed examples are presented by way of illustration of certain specific embodiments of the invention. The examples are representative only and should not be construed as limiting the present invention in any aspect.

Example 1.

Preparation of Ile-Gln-Leu-Met-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-Arg-Arg-Arg-Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala-NH_2 ([Ile^5, Met^8]-hPTHrP (5-34)-NH_2; Compound No. 15 in Table 2):

This peptide was synthesized on 1% cross-linked 4-methylbenzhydrylamine polystyrene solid phase carrier (amino group content 0.5 mmol) in accordance with the solid phase synthetic method as previously mentioned. The following amino acid derivatives were used for preparing this peptide.

Boc-Ala, Boc-Asp (OcHx), Boc-Asn, Boc-Arg (Tos), Boc-Gly, Boc-Glu (OcHx), Boc-Gln, Boc-His (Bom), Boc-Ile, Boc-Leu, Boc-Lys (Cl-Z), Boc-Met, Boc-Phe, Boc-Ser (Bzl), Boc-Thr (Bzl).

Elongation of the peptide chain was performed by repeating the procedure as shown in Table 1.

Table 1

Treating method	Times treated	Period (min)
Deprotection		
1. Wash with CH_2Cl_2	1	1.0
2. Wash with 50% CF_3COOH/CH_2Cl_2	1	5.0
3. Deprotect with 50% CF_3COOH/CH_2Cl_2	1	25
4. Wash with CH_2Cl_2	1	1.0
Neutralization		
1. 10% diisopropylethylamine/CH_2Cl_2	2	2.0
2. Wash with CH_2Cl_2	2	5.0
3. Wash with dimethylformamide	5	1.0
Condensation*		
1. sym-acid anhydride of tert-Butoxy-carbonylamino acid(2 mol equiv)**/ dimethylformamide	1	30-60
2. Wash with dimethylformamide	5	1.0
3. Wash with CH_2Cl_2	5	1.0

* Boc-Asn, Boc-Gln, Boc-Arg (Tos) and Boc-His (Bom) were subjected to DCC/HOBt method [Mojsov et al., J. Org. Chem., 45, 555 (1980)]. Boc-Asn, Boc-Gln, Boc-Arg (Tos), Boc-Ile, Boc-Thr (Bzl) and Boc-His (Bom) were subjected to condensation twice.

** sym-Acid anhydride obtained by mixing the protected amino acids with DCC was used without isolation.

Removal of the solid carrier and protecting group was performed by known HF method. More particularly, the protected peptide bound to polystyrene was reacted with a mixture of 10% p-cresole, 5% dimethylsulfide, and 85% dry hydrogen fluoride at 0 °C for one hour, and the reaction mixture was concentrated under reduced pressure. The residue was washed with ethyl acetate and extracted with 1 M acetic acid. The extract was lyophilized to give crude peptide. The crude peptide was subjected to reversed phase high performance liquid chromatography and eluted with 0.1% trifluoroacetic acid-acetonitrile in linear gradient. After lyophilization of relevant fractions, the partially purified peptide was subjected to CM Toyopearl 650S (1.5 x 20 cm) in 20 mM ammonium acetate (pH 6.0) and eluted with 20 mM-1.0 M ammonium acetate in linear gradient of ion concentration. The fractions containing the peptide was lyophilized, subjected to gel filtration with Sephadex G 25 (1.5 x 40 cm), which was equilibrated with 2% acetic acid, for conversion into the acetate and lyophilized to give the pure product.

Yield: 40.0 mg

Amino acid analysis: The peptide sample was hydrolyzed in 5.5 M hydrochloric acid at 110°C for 48 hours and subjected to the amino acid analysis. The data within the parenthesis below represent calculated values. Correction for compensating possible decomposition of amino acids during the hydrolysis was not made.

Asp: 1.97 (2), Thr: 0.93 (1), Ser: 0.80 (1), Glu: 3.11 (3),
Gly: 1.02 (1), Ala: 2.10 (2), Met: 0.94 (1), Ile: 3.74 (4),
Leu: 3.98 (4), Phe: 2.06 (2), Lys: 1.91 (2), His: 3.79 (4),
Arg: 3.27 (3)

Optical Rotation [α_D^{25} : -57.4° (C = 0.1, 1 M AcOH)

The following peptides listed in Table 2 were synthesized in the same method as in Example 1.

Table 2

Compound	AAA	BBB	CCC	DDD	EEE	FFF	GGG	HHH	III	JJJ	Optical Rotation $[\alpha]_D^{25}$ (c 0.1)
1	Ser	Glu	Ile	Gln	Leu	Met	Ala	Leu	His	His	-61.3°
2	Ser	Glu	Ile	Gln	Leu	Met	Gln	Leu	His	His	-56.9°
3	Ser	Glu	Ile	Gln	Leu	Met	Leu	Leu	His	His	-56.7°
4	Ser	Glu	Ile	Gln	Leu	Met	Ser	Leu	His	His	-68.5°
5	Ser	Glu	Ile	Gln	Leu	Met	Asn	Glu	His	His	-54.0°
6	Ser	Glu	Ile	Gln	Leu	Leu	Asp	Lys	His	His	-63.8°
7	-	-	D-aIle	Gln	Leu	Met	Asp	Lys	His	His	-58.1°
8	-	-	Phe	Gln	Leu	Met	Asp	Lys	His	His	-52.2°
9	-	-	Leu	Gln	Leu	Met	Asp	Lys	His	His	-62.1°
10	-	-	-	Myr-Gln	Leu	Met	Asp	Lys	His	His	-46.7°
11	-	-	Cha	Gln	Leu	Met	Asp	Lys	His	His	-78.3°
12	-	-	Ile	Gly	Leu	Met	Asp	Lys	His	His	-44.0°
13	-	-	Ile	Gln	Phe	Met	Asp	Lys	His	His	-64.4°
14	-	-	Ile	Gln	Nle	Met	Asp	Lys	His	His	-66.0°

Table 2 (continued)

Compound	AAA	BBB	CCC	DDD	EEE	FFF	GGG	HHH	III	JJJ	Optical Rotation $[\alpha]_D^{25}$ (c 0.1)
15	-	-	Ile	Gln	Leu	Met	Asp	Lys	His	His	-57.4°
16	-	-	Ile	Gln	Leu	Nle	Asp	Lys	His	His	-62.5°
17	-	-	Ile	Gln	Leu	Met	Ala	Lys	His	His	-69.9°
18	-	-	Ile	Gln	Leu	Met	Ala	Leu	His	His	-61.5°
19	-	-	Ile	Gln	Leu	Met	Asp	Lys	Arg	His	-52.0°
20	-	-	Ile	Gln	Leu	Met	Asp	Lys	His	Lys	-61.8°
21	-	-	Ac-Ile	Gln	Leu	Met	Asp	Lys	His	His	-74.9°
22	-	-	Myr-Ile	Gln	Leu	Met	Asp	Lys	His	His	-44.6°
23	-	-	Mpa-Ile	Gln	Leu	Met	Asp	Lys	His	His	-61.3°
24	-	-	Cap-Ile	Gln	Leu	Met	Asp	Lys	His	Lys	-54.5°
25	-	-	Ac-Lys(Myr)	Gln	Leu	Met	Asp	Lys	His	Lys	-47.0°
26	-	-	Ac-Lys(Hex)	Gln	Leu	Met	Asp	Lys	His	Lys	-61.9°
27	-	-	Ac-Lys(Cap)	Gln	Leu	Met	Asp	Lys	His	Lys	-49.5°
28	-	-	Ac-Lys(Pal)	Gln	Leu	Met	Asp	Lys	His	Lys	-39.0°

a: Measured in 1M acetic acid. "c" in the parenthesis denotes the weight of the peptide contained. The peptide content was determined by amino acid analysis.

Experiment 1. Determination of PTH antagonism

PTH antagonism of the PTHrP derivatives (I) of the present invention was determined on the basis of the output of cAMP, using cultured mouse osteoblast MC3T3-E1.

To a multiwellculture plate of 12-well were inoculated 1 x 10^5 cells/well of the cultured cells, and the

plate was incubated at 37° C under 95% air-5% CO_2 atmosphere for 3 days after addition of α-modified MEM containing 10% semi-fetal bovine serum as a medium. Then, the medium was exchanged with α-modified MEM containing 1% bovine serum albumin and the cultured cells were incubated for 6 hours. The medium was replaced by α-modified MEM containing various concentrations of the compound of the present invention, 5×10^{-9} M hPTH (1-34), 1% bovine serum albumin and 1 mM isobutylmethylxanthin, and the mixture was incubated for 1 hour. The medium was separated from the cells, and the medium was used as a sample for assaying cAMP. The cells were shaken with 90% n-propyl alcohol to extract cAMP according to Yamaguchi et al., J. Biol. Chem., 262, 7711 -7718 (1987) for preparing another sample.

Assay of cAMP was performed using a commercially available cAMP-radioimmunoassay kit. Tables 3 and 4 below show 50% inhibition (IC_{50}) of the output of cAMP due to the compound of the present invention, when the amount of cAMP produced by 5×10^{-9} M hPTH (1-34) is regarded as 100%. Compound numbers in the tables correspond to those in Table 2. [Tyr^{34}]-hPTH (3-34)-NH_2 and hPTHrP (3-34)-NH_2, both of which are heretofore known as a PTH antagonist, were used as active controls in Table 3, while Compound No. 15 was used as a control in Table 4.

Table 3

Compound	IC_{50} Ratio
(Tyr34)-hPTH(3-34)-NH_2	1
hPTHrP(3-34)-NH_2	0.087
Compound No. 1	0.003
Compound No. 2	0.003
Compound No. 8	0.026
Compound No. 15	0.017

Table 4

Compound	IC_{50} Ratio
(Leu11,D-Trp12)-hPTHrP(7-34)-NH_2	3.91
Compound No. 9	1.02
Compound No. 10	2.24
Compound No. 14	0.922
Compound No. 15	1
Compound No. 16	0.930
Compound No. 17	1.17
Compound No. 18	1.97
Compound No. 19	0.934
Compound No. 20	1.01
Compound No. 21	0.934
Compound No. 22	0.765
Compound No. 25	0.343

As shown in Table 3 above, Compound No. 1 of the present invention showed 50% inhibition at a concentration of below about 1/300 of [Tyr^{34}]-hPTH (3 -34)-NH_2 and at a concentration of about 1/29 of hPTHrP (3-34)-NH_2 when MC3T3-E1 was used.

Thus, the PTHrP derivatives (I) of the present invention are useful as a therapeutic agent for treating dysbolism associated with calcium or phosphoric acid, such as hypercalcemia, osteoporosis, hyperparathyroidism, renal osteodystrophy, and the like, and other related diseases involving PTH or PTHrP.

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO:1

(A) LENGTH: 32 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 32-Xaa means Ala modified at the carboxy terminal with an amino group.

Ser Glu Ile Gln Leu Met His Ala Leu Gly Lys Ser Ile Gln Asp Leu
1 5 10 15

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:2

(A) LENGTH: 32 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 32-Xaa means Ala modified at the carboxy terminal with an amino group.

Ser Glu Ile Gln Leu Met His Gln Leu Gly Lys Ser Ile Gln Asp Leu
1 5 10 15

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:3

(A) LENGTH: 32 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 32-Xaa means Ala modified at the carboxy terminal with an amino group.

Ser Glu Ile Gln Leu Met His Leu Leu Gly Lys Ser Ile Gln Asp Leu
1 5 10 15

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:4

(A) LENGTH: 32 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 32-Xaa means Ala modified at the carboxy terminal with an amino group.

Ser Glu Ile Gln Leu Met His Ser Leu Gly Lys Ser Ile Gln Asp Leu
1 5 10 15

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:5

(A) LENGTH: 32 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 32-Xaa means Ala modified at the carboxy terminal with an amino group.

Ser Glu Ile Gln Leu Met His Asn Glu Gly Lys Ser Ile Gln Asp Leu
1 5 10 15

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:6

(A) LENGTH: 32 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 32-Xaa means Ala modified at the carboxy terminal with an amino group.

Ser Glu Ile Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu
1 5 10 15

Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:7

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means D-alle.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:8

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Phe Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:9

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Leu Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:10

(A) LENGTH: 29 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means Myristoyl Gln.
(2) 29-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg Arg
1 5 10 15

Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25

INFORMATION FOR SEQ ID NO:11

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means β-Cyclohexyl Ala.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:12

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Ile Gly Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:13

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

```
Ile Gln Phe Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
 1               5                  10                  15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
            20                  25                  30
```

INFORMATION FOR SEQ ID NO:14

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 3-Xaa means Norleucine.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

```
Ile Gln Xaa Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
 1               5                  10                  15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
            20                  25                  30
```

INFORMATION FOR SEQ ID NO:15

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Ile Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:16

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 4-Xaa means Norleucine.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Ile Gln Leu Xaa His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:17

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Ile Gln Leu Met His Ala Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:18

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Ile Gln Leu Met His Ala Leu Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:19

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Ile Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu Arg His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:20

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Ile Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His Lys Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:21

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means Acetyl Ile.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:22

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means Myristoyl Ile.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:23

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means 3-Methylpentanoyl Ile.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:24

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means n-Decanoyl Ile.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His Lys Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:25

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means Acetyl ϵ-Myristoyl Lys.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His Lys Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:26

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means Acetyl ϵ-Hexanoyl Lys.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His Lys Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:27

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means Acetyl ϵ-Decanoyl Lys.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His Lys Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

INFORMATION FOR SEQ ID NO:28

(A) LENGTH: 30 amino acids

(B) TYPE: peptide

(C) TOPOLOGY: linear

(D) FEATURES: (1) 1-Xaa means Acetyl ϵ-palmitoyl Lys.
(2) 30-Xaa means Ala modified at the carboxy terminal with an amino group.

Xaa Gln Leu Met His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg
1 5 10 15

Arg Phe Phe Leu His Lys Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

Claims

1. Peptide derivatives of the general formula (I):

AAA BBB CCC DDD EEE FFF His GGG HHH Gly Lys Ser Ile Gln Asp Leu
1 5 10 15

Arg Arg Arg Phe Phe Leu III JJJ Leu Ile Ala Glu Ile His Thr Xaa
20 25 30

... (I)

wherein AAA is deletion or Ser, BBB is deletion or Glu, CCC is deletion, Ile, Phe, Leu, cyclohexylalanine, D-alle or Lys substituted at the ϵ-position by C_6-C_{18} alkylcarbonyl group, DDD is Gly or Gln,

EEE is Leu, Nle or Phe, FFF is Met, Leu or Nle, GGG is Ala, Ser, Leu, Asn, Asp or Gln, HHH is Leu, Glu or Lys, III is His, Lys or Arg, JJJ is His, Lys or Arg, Xaa is Ala modified at the carboxy terminal with an amino group, provided that CCC and DDD may independently be modified at the amino terminal by C_2-C_{18} alkylcarbonyl group, and that EEE is not Leu when FFF is Met, GGG is Asn, HHH is Leu, III and JJJ are His, and the salts thereof.

2. The peptide derivative of Claim 1 wherein AAA and BBB are deletion, CCC is Ile, DDD is Gln, EEE is Leu, FFF is Met, GGG is Asp, HHH is Lys, III and JJJ are His.

3. The peptide derivatives of Claim 1 wherein AAA and BBB are deletion, CCC is Ile modified at the amino terminal by C_2-C_{18} alkylcarbonyl group or Lys substituted at the ϵ-position by C_6-C_{18} alkylcarbonyl group, DDD is Gln, EEE is Leu, FFF is Met, GGG is Asp, HHH is Lys, III is His, JJJ is His or Lys.

4. The use of the peptide derivatives according to previous claims 1 to 3 for the preparation of a pharmaceutical useful as a parathyroid hormone antagonist.

Claims for the following Contracting States: ES GR

1. A process for the preparation of a polypeptide derivatives, characterized by synthesizing of amino acids or protected amino acids or peptide fragments to obtain a peptide derivative of the general formula (I)

```
AAA BBB CCC DDD EEE FFF His GGG HHH Gly Lys Ser Ile Gln Asp Leu
 1               5                  10                  15

Arg Arg Arg Phe Phe Leu III JJJ Leu Ile Ala Glu Ile His Thr Xaa
            20                  25                  30

                                                        ... (I)
```

wherein AAA is deletion or Ser, BBB is deletion or Glu, CCC is deletion, Ile, Phe, Leu, cyclohexylalanine, D-alle or Lys substituted at the ϵ-position by C_6-C_{18} alkylcarbonyl group, DDD is Gly or Gln, EEE is Leu, Nle or Phe, FFF is Met, Leu or Nle, GGG is Ala, Ser, Leu, Asn, Asp or Gln, HHH is Leu, Glu or Lys, III is His, Lys or Arg, JJJ is His, Lys or Arg, Xaa is Ala modified at the carboxy terminal with an amino group, provided that CCC and DDD may independently be modified at the amino terminal by C_2-C_{18} alkylcarbonyl group, and that EEE is not Leu when FFF is Met, GGG is Asn, HHH is Leu, III and JJJ are His, and the salts thereof.

2. The process according to claim 1, wherein AAA and BBB are deletion, CCC is Ile, DDD is Gln, EEE is Leu, FFF is Met, GGG is Asp, HHH is Lys, III and JJJ are His.

3. The process according to claim 1, wherein AAA and BBB are deletion, CCC is Ile modified at the amino terminal by C_2-C_{18} alkylcarbonyl group or Lys substituted at the ϵ-position by C_6-C_{18} alkylcarbonyl group, DDD is Gln, EEE is Leu, FFF is Met, GGG is Asp, HHH is Lys, III is His, JJJ is His or Lys.

4. The use of the peptide derivatives as obtained by the processes according to claims 1 to 3 for the preparation of a pharmaceutical useful as a parathyroid hormone antagonist.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number EP 91105881.6

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	EP - A2 - 0 341 962 (MERCK & CO.INC.) * Totality * --	1-4	C 07 K 7/10 C 07 K 1/04 A 61 K 37/02 A 61 K 37/43
D,Y	EP - A2 - 0 341 963 (MERCK & CO.INC.) * Totality * --	1-4	
A	EP - A2 - 0 293 160 (MERCK & CO.INC.) * Claims 1,5 * --	1-4	
A	EP - A2 - 0 293 158 (MERCK & CO.INC.) * Claims 1,5 * --	1-4	
A	CHEMICAL ABSTRACTS, vol. 105, No. 25, December 25, 1986, Columbus, Ohio, USA M. ROSENBLATT "Peptide hormone antagonists that are effective in vivo. Lessons from parathyroid hormone" page 82, right column, abstract-No. 218 917n & N.Engl.J.Med. 1986,315(16), 1004-13 (Eng). ----	1-4	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 K
A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	18-07-1991	AUGUSTIN

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)